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SPECIFICATION

~~UCP-2 Promoter DNA~~
UCP-2 Promoter And Use Thereof

FIELD OF THE INVENTION

5 This invention relates to a novel promoter for
gene expression and its use. Specifically, this
invention relates to a DNA containing the promoter
region of human uncoupling protein-2 (UCP-2) gene, a
transformant transformed with the said DNA, and a
10 method for screening a compound or its salt that
promotes or inhibits UCP-2 promoter activity.

BACKGROUND OF THE INVENTION

15 Uncoupling protein (UCP) is a proton transporter
present in the mitochondrial inner membrane. Since UCP
changes intracellular energy stored as fat to heat
without using other energy consuming processes, UCP is
considered to play an important role in maintenance of
body temperature in homeothermal animals. Because of
20 this function, UCP is considered to be an important
factor that determines the efficiency of energy
metabolism in homeothermal animals.

25 Three molecular species of uncoupling protein have
been identified to date, and are called uncoupling
proteins-1 (UCP-1), -2 (UCP-2 or UCPH), and -3 (UCP-3).

30 UCP-1, the first isolated among the uncoupling
protein family, is specifically expressed in brown fat
cells (Line, C.S. and Klingenberg, M. (1980), FEBS
Lett., 113, 299-303; Jacobsson, A. et al. (1985), J.
Biol. Chem., 260, 16250-16254; Bouillaud, F. et al.
(1986), J. Biol. Chem., 261, 1487-1490). UCP-2 was
isolated as a homologue of UCP-1, and confirmed to be
widely expressed in various organs (Gimeno, R.E. et al.
(1997), Diabetes, Vol. 46, 900-906; Fleury, C., et al.
35 (1997), Nature Genet., Vol. 15, 269-272). UCP-3 was

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isolated as a UCP specifically expressed in muscle (Vidal-Puig, A. et al. (1997), Biochem. Biophys. Res. Commun., Vol. 235, No. 1, 79-82; Boss, O. et al. (1997), FEBS Lett., 408, 33-38).

5 Generally, UCP-1 is considered to play an important role in maintenance of body temperature in rodents and hibernants. As for the basis, the number of brown fat cells that mainly express UCP-1 is lower in large sized animals and animal species inhabiting in
10 relatively warm weather (Rothwell, N.J. and Stock, M.J. (1979), Nature, Vol. 281, 31-35). Thus, in these animals including human, UCP-2 or UCP-3, not UCP-1, may mainly be responsible for the control of the normal body temperature maintenance system and energy
15 consuming process (Hosoda, K. et al. (1998), Obesity Research (Himan Kenkyu), Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), Nature, Vol. 387, 90-93).

Therefore, it may be possible to adjust the energy consumption/accumulation balance by controlling the
20 gene expression or activity of UCP-2 or UCP-3 in these animals including human (Hosoda, K. et al. (1998), Obesity Research (Himan Kenkyu), Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), Nature, Vol. 387, 90-93). In human, enhancement of energy consumption is
25 considered to promote consumption of not only dietary energy but also energy accumulated as fat. Accordingly, a decrease of body fat in human may lead to improvement of obesity, the major cause of lifestyle diseases which become a problem in developed countries in recent years
30 (Fleury, C. et al. (1997), Nature Genetics, Vol. 15, 269-272).

UCP-2 is also considered to be the major cause of fever observed in immunological inflammation such as infection, and inhibition of UCP-2 gene activity may
35 reduce fever in immunological inflammation (Shigenaga,

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F.R. et al. (1998), Biochim. Biophys. Res. Commun., Vol. 244, No. 1, 75-78).

In animals, especially in higher animals, organs differentiate and mature upon biogenesis, and develop to exert each function. During this process, various organ-specific proteins are transiently or constantly expressed and provide the organ-specificity.

The general gene expression control system in animals includes the transcription induction system (promoter, enhancer). Promoter regions are generally located adjacent to the 5' upstream region of base sequences on chromosomes that are normally transcribed into messenger RNAs. Transcriptional regulator protein is bound to or dissociated from base sequence generally called regulator sequence in promoter regions, by which the transcription level of genes located downstream of the 3' region is regulated. Therefore, the transcriptional gene expression level can be estimated from the promoter activity to some extent. It is also known that the base sequences located downstream of the 3' region of a promoter do not affect the promoter activity in most cases. Therefore, promoter activity can be readily measured by substituting the transcribed messenger RNA for a base sequence encoding a protein with detectable enzyme activity (reporter). Recent technical innovation has made measurement of promoter activity using reporters very sensitive and simple, and measurement of promoter activity is used in drug screening and analysis of biological function.

For example, transcriptional regulatory factors of fat cell differentiation include peroxisome proliferation-activated receptor γ (PPAR γ) (Tontontz, P. et al. (1995), Curr. Opin. Genet. Dev., Vol. 5, 571-576), retinoid X receptor (RXR), CCAAT/enhancer binding protein (C/EBP) (Cornelius, P., et al. (1994), Annu.

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Rev. Nutr. Vol. 14, 99-129), etc. The transcriptional regulation by these factors are closely involved in the gene expression related to fat cells. It has been reported that the promoter regions of fat cell-related genes including UCP-2 gene contain the binding sequences for these transcriptional regulatory factors (regulator sequences). These sequences in promoters are considered to play important roles in the actual regulation of UCP-2 transcription in vivo.

Accordingly, substances that enhance expression of UCP-2 or UCP-3 gene and protein may be used as antiobestic drugs that reduce body fat content. UCP-2 is also considered to be the major cause of fever in immunological inflammation observed in infection, and substances that inhibit UCP-2 gene activity may reduce fever in immunological inflammation.

If a cell line expressing an appropriate reporter gene connected to the promoter region described above is established, the cell line may be used for screening a drug that promotes or inhibits the UCP-2 expression. In screening substances that may be used as antiobestic drugs, responses more similar to those in vivo can be obtained by including these regulator sequences in the promoter-reporter system, which is very advantageous in screening human antiobestic drugs.

However, human UCP-2 promoter containing the regulator sequence has not yet been identified, and no simple screening method using the promoter described above has been available for substances that affect the human UCP-2 gene expression.

DISCLOSURE OF THE INVENTION

The inventors performed extensive studies, and successfully obtained the human genomic UCP-2 gene using human UCP-2 cDNA fragments as probes in attempt

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to establish a screening method for searching substances that affect the human UCP-2 gene expression. The gene was digested with restriction enzymes, and 6.5 kb DNA of the upstream region containing a part of the structural gene encoding UCP-2 was obtained. From the DNA obtained, 3.5 kb DNA containing the base sequence deduced to be the 1st and 2nd exons (2.5 kb DNA as the 5' upstream region) were re-cloned in plasmid DNA.

A plasmid DNA was constructed by connecting luciferase gene as a reporter gene to downstream of the 3.5 kb DNA. Measuring the luciferase activity in transformants of HepG2 cells and MG-63 cells differentiated to fat cell-like cells, UCP-2 promoter was found in the 3.3 kb DNA of the upstream region of the UCP-2 structural gene. As a result of detailed analysis, the regulator sequence that may control the expression of UCP-2 was found.

The inventors proceeded the study based on these findings, and completed the present invention. The present invention relates to the followings:

- (1) A DNA containing uncoupling protein-2 (UCP-2) promoter region containing the regulator sequence;
- (2) A DNA described in (1) wherein the regulator sequence is a sequence containing peroxisome proliferator response element (PPRE);
- (3) A DNA described in (1) wherein the regulator sequence is a sequence containing CCAAT/enhancer binding protein (C/EBP) binding sequence;
- (4) A DNA described in (1) wherein the promoter region is a base sequence presented by position 1 to 2270 of SEQ ID NO: 1 or a base sequence containing a part of the said base sequence;
- (5) A recombinant vector containing a DNA described in (1);
- (6) A recombinant vector described in (5) containing a

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DNA having a structural gene under control of UCP-2 promoter region containing a regulator sequence;

(7) A transformant transformed by a recombinant vector described in (5);

5 (8) A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in (7);

(9) A method for screening a compound or its salt that promotes or inhibits heat production characterized by use of a transformant described in (7);

10 (10) A method for screening an antiobestic drug, an antidiabetic drug, a depressor, an antihyperlipemic drug, and an antipyretic drug characterized by use of a transformant described in (7);

15 (11) A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in (7);

20 (12) A compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using a screening method described in (8) or a screening kit described in (11);

25 (13) A compound or its salt that promotes or inhibits heat production obtained using a screening method described in (9); and

(14) A pharmaceutical composition containing a compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using a screening method described in 30 (8) or a screening kit described in (11).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in 35 Example 1 (continued to Figure 2).

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 Figure 2 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 1 to Figure 2).

Figure 3 shows the base sequence of cDNA
 5 containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 2 to Figure 4).

Figure 4 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 3 to Figure 5).

10 Figure 5 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 4 to Figure 6).

Figure 6 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in
 15 Example 1 (continued from Figure 5).

Figure 7 shows the luciferase activity measured in Example 2.

Figure 8 shows the luciferase activity measured in Example 3.

20 Figure 9 shows the structure of the UCP-2 promoter deficient-clones constructed in Example 4. The numbers in the Figure represent the base number starting from the transcription initiation site.

Figure 10 shows the promoter activity measured in
 25 Example 4.

BEST MODE OF EMBODIMENT OF THE INVENTION

A DNA containing the UCP-2 promoter region containing the regulator sequence of this invention may
 30 be any DNA containing the regulator sequence described below with UCP-2 promoter activity.

Specifically, a DNA of this invention may be any DNA containing the base sequence presented by position
 1 to 2270 of SEQ ID NO: 1 or a part of said sequence.

35 A DNA of this invention may be genomic DNA, cDNA,

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epidermal cells, epithelial cells, endothelial cells, fibroblasts, fibre cells, muscle cells, fat cells, immune cells (e.g. macrophages, T cells, B cells, natural killer cells, mast cells, neutrophils, basophils, eosinophils, monocytes), megakaryocytes,

25 Specifically, a recombinant DNA containing the
human UCP-2 promoter region of this invention can be
obtained as follows.

Using the base sequence corresponding to the previously reported amino acid sequences of human UCP cDNA (Fleury, C. et al. (1997), Nature Genet. Vol. 15, 269-272) as the probes, for example, human genomic library inserted in EMBL3 vector is screened by publicly known method, and λ phage clones that react with the probes are obtained. A DNA is extracted from these phage clones, and the restriction enzyme map of

the human gene inserted in the clones is prepared. DNA fragments are prepared by digestion with restriction enzymes, and the fragments react with the probes for the most upstream region of the cDNA are re-cloned in
5 vectors for animal cells such as pCD vector, cDM8 vector (Aruffo, A. and Seed, B. (1987), Proc. Natl. Acad. Sci. USA, 84, 8573-8577), and retrovirus vector (Cone, R.D. and Mulligan, R.C. (1984), Proc. Natl. Acad. Sci. USA, 81, 6349-6353), and *Escherichia coli* plasmids
10 such as pUC vector (Vieira, J. and Messing, J. (1987), Methods in Enzymology, 153, 3-11), and PCR-blunt vector (Ausubel, F.M. et al. (1994), Current Protocols in Molecular Biology), but not limited to these vectors. The base sequences of the cloned DNA are determined,
15 and the position of the translation initiation codon on the gene can be determined by, for example, comparing the base sequence with the cDNA sequence. The position of the transcriptional initiation site on the gene can also be determined by comparing the base sequence with
20 the 5' end of known cDNA. By investigating motifs in the entire sequence, the binding site of known transcriptional regulatory factors can be determined.

The obtained DNA can be used without modification or if necessary, after digestion with restriction
25 enzymes or being bound by linkers.

To measure the promoter activity, a detectable structural gene may be connected in downstream of the promoter region. For the structural gene connected in downstream of the promoter region, various reporter
30 genes are used. For the reporter gene, luciferase gene, chloramphenicol acetyltransferase (CAT) gene, alkaline phosphatase gene, and β -galactosidase gene are commonly used, but any other structural genes for which a method of detecting the gene product is available may be used.
35 To insert the above structural gene into the vector,

the structural gene is ligated to an appropriate restriction enzyme site located downstream of the promoter region in the correct transcriptional orientation.

5 For the host transformed by the recombinant vector described above, for example, *Escherichia* genus, *Bacillus* genus, Yeast, insect cells, insects, and animal cells are used.

Specific examples of the host *Escherichia* genus
10 are *Escherichia coli* K12·DH1 [Proceedings of the National Academy of Sciences of the USA (Proc. Natl. Acad. Sci. USA), Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JM109, JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)],
15 HB101 [Journal of Molecular Biology, Vol. 41, 459 (1969)], and C600 [Genetics, Vol. 39, 440 (1954)].

For the host *Bacillus* genus, for example, *Bacillus subtilis* MI114 [Gene, Vol. 24, 255 (1983)] and 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)] are used.

20 For the host yeast, for example, *Saccharomyces cerevisiae* AH22, AH22R⁺, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYC1913, NCYC2036, and *Pichia pastoris* are used.

For the host insect cells, for example, when the
25 virus is AcNPV, *Spodoptera frugiperda* cells (Sf cells), MG1 cells derived from the middle gut of *Trichoplusia ni*, High FiveTM cells derived from *Trichoplusia ni* eggs, *Mamestra brassicae*-derived cells, and *Estigmena acrea*-derived cells are used. When the virus is BmNPV,
30 silkworm-derived cell line *Bombyx mori* N (BmN cells) are used. For said Sf cells, for example, Sf9 cells (ATCC CRL1711), Sf21 cells (Vaughn, J.L. et al., *In Vivo*, 13, 213-217 (1977)) are used.

For the host insect, for example, silkworm larvae
35 are used [Maeda et al., *Nature*, Vol. 315, 592 (1985)].

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Animal cells can be transformed by, for example,

the methods described in Cell Engineering (Saibo Kogaku), Separate Vol. 8, New Cell Engineering Experimental Protocol, 263-267 (1995) (Shujun-sha) and Virology, Vol. 52, 456 (1973).

5 The transformant described above is cultured in the presence of the specified compound, and by measuring and comparing the gene product in the cultured material, the ability of controlling the promoter activity of the compound can be examined.

10 The transformant is cultured by publicly known
methods. For the medium for culturing the transformant
using *Escherichia* and *Bacillus* hosts, liquid medium is
appropriate, which contains carbon source, nitrogen
source, inorganic compounds, and other substances
15 necessary for the growth of the transformants. The
carbon source includes, for example, glucose, dextrin,
soluble starch, and sucrose, etc. The nitrogen source
includes, for example, inorganic and organic compounds
such as ammonium salts, nitrates, cornsteep liquor,
20 peptone, casein, meat extract, soybean cake, and potato
extract, etc. The inorganic compounds include, for
example, calcium chloride, sodium dihydrogen phosphate,
and magnesium chloride, etc. Yeast extract, vitamins,
and growth-stimulating factors may be added. The pH
25 about 5 - 8 is desirable for the culture medium.

For the culture medium for bacteria of *Escherichia* genus, for example, M9 medium containing glucose and casamino acid (Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972) is preferred. When a higher efficiency of the promoter is required, reagent such as 3- β -indolylacrylic acid may be added. When the host is bacteria of *Escherichia* genus, the bacteria are generally cultured at about 15 - 43°C for about 3 - 24 hours, and aeration or stirring may be added to the

culture if necessary.

When the host is bacteria of *Bacillus* genus, the bacteria are generally cultured at about 30 - 40°C for about 6 - 24 hours, and aeration or stirring may be added to the culture if necessary.

For the medium for culturing the transformant in yeast host, for example, Burkholder minimum medium [Bostian, K.L. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)] and SD medium containing 0.5% casamino acid [Bitter, G.A. et al., Proc. Natl. Acad. Sci. USA, Vol. 81, 5330 (1984)] are used. The pH of the medium is preferably adjusted to about 5 - 8. The culture conditions are generally about 20 - 35°C for about 24 - 72 hours, and aeration or stirring may be added to the culture if necessary.

For the medium for culture of the transformants in insect cells and insect hosts, Grace's insect medium [Grace, T.C.C., Nature, 195, 788 (1962)] containing appropriate supplements such as inactivated 10% bovine serum is used. The pH of the medium is preferably adjusted to about 6.2 - 6.4. Usually, the culture conditions are at about 27°C for about 3 - 5 days, and aeration or stirring may be added to the culture if necessary.

For the culture medium of the transformants in animal cell hosts, for example, MEM containing about 5 - 20% fetal calf serum [Science, Vol. 122, 501 (1952)], DMEM [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, Vol. 199, 519 (1967)], and 199 medium [Proceeding of the Society for the Biological Medicine, Vol. 73, 1 (1950)] are used. The pH is preferably adjusted to about 6 - 8. Usually, the culture conditions are about 30 - 40°C for about 15 - 60 hours, and aeration or stirring may be added to the culture if necessary.

Specifically, the regulator sequence may be any sequence of the base sequence presented by position from 1 to 2270 of SEQ ID NO: 1 to which the UCP-2 transcriptional regulatory factor can bind, such as

5 sequences containing peroxisome proliferator response element (PPRE) presented by position 284 to 296 of SEQ ID NO: 1, sequences containing CCAAT/enhancer binding protein (C/EBP) binding sequence presented by position 1316 to 1320, 1364 to 1368, or 1698 to 1692 of SEQ ID

10 NO: 1, sequences containing glucocorticoid response element (GRE) presented by position 753 to 758, 1023 to 1030, or 1450 to 1455 of SEQ ID NO: 1, and sequences containing MyoD presented by position 1428 to 1437 of SEQ ID NO: 1.

15 Therefore, a DNA of this invention contains the promoter region containing the said regulator sequence, and a DNA of this invention may contain a multiple number of the said regulator sequences.

For the base sequences containing a part of the

20 base sequence presented by position 1 to 2270 of SEQ ID NO: 1, any base sequences containing the regulator sequence described above may be used. Specifically, the base sequence presented by position 255 to 430 of SEQ ID NO: 1, the base sequence presented by position

25 255 to 717 of SEQ ID NO: 1, the base sequence presented by position 717 to 1133 of SEQ ID NO: 1, the base sequence presented by position 1133 to 1389 of SEQ ID NO: 1, and the base sequence presented by position 255 to 1857 of SEQ ID NO: 1 are used.

30 Furthermore, the base sequence presented by position 571 to 2270 of SEQ ID NO: 1, the base sequence presented by position 717 to 2270 of SEQ ID NO: 1, the base sequence presented by position 1133 to 2270 of SEQ ID NO: 1, the base sequence presented by position 1389

35 to 2270 of SEQ ID NO: 1, and the base sequence

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Since a DNA of this invention contains the UCP-2 promoter region containing the regulator sequence,

(1) A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production)

A method for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention is characterized by measuring and comparing polypeptide expression between a transformant of this invention contacted to test compound and the transformant lacking the UCP-2 promoter region of this invention contacted to the test compound.

For the polypeptide to be expressed, polypeptides encoded by the structural genes described above (preferably reporter genes) are used.

For the measurement method of polypeptide
35 expression, for example, measurement of luciferase

activity according to the method described by Brasier, A.R. et al. (1989) in Biotechniques Vol. 7, 1116-1122, is used.

- (2) A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production)

A kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production) is characterized by use of the transformant described above. Examples of the kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention are as follows.

① Screening reagents

1. Cell culture medium

Dulbecco's modified Eagle's medium (Gibco Co.) supplemented with 10% fetal calf serum (Gibco Co.)

2. Cell differentiation medium

Dulbecco's modified Eagle's medium (Gibco Co.) supplemented with 5% rabbit serum (Gibco Co.)

3. Plasmid for measurement of UCP-2 promoter activity

pGL3-basic (Promega Co.) plasmid DNA carrying UCP-2 promoter sequence of this invention and a structural gene (e.g. luciferase gene) inserted downstream of the

- UCP-2 promoter

4. Host cell line

MG-63 cells (osteosarcoma cell line, obtained from ATCC)

5. Test compounds

Aqueous solutions are stored at 4°C or -20°C, and diluted to 1 μ M with cell differentiation medium at use. Test compounds that are slightly soluble in water are dissolved in dimethylformamide, DMSO, and methanol.

② Screening method

- Host cells are seeded in 96-well microplates at a

density of 1×10^5 cells/well, and cultured in an incubator at 37°C in 5% CO₂ overnight.

The cells are transfected with 1 µg/well of plasmid for UCP-2 promoter activity measurement.

5 One hour after transfection, 0.1 ml of test compound is added to each well, and the cells are cultured in an incubator at 37°C in 5% CO₂ for 48 hours.

After culture, 0.1 ml of PicaGene LT (Toyo Ink Co.) is added to each well, stirred for five minutes,
10 and then the luminescence is measured using a 96-plate measurement system (Amersham-Pharmacia Co.).

(3) A compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production) obtained using the
15 screening method described in (1) and the screening kit described in (2)

If a compound that promotes or inhibits UCP-2 promoter activity is found using the screening method described in (1) or the screening kit described in (2),
20 the compound may be used as a prophylactic or therapeutic drug for obesity syndrome because the compound increases or promotes heat production, and thus, the compound may be used as a radical therapeutic drug for lifestyle diseases (diabetes, hypertension,
25 hyperlipidemia). Therefore, the compound may be used as an antiobestic drug, an antidiabetic drug, a depressor, and an antihyperlipemic drug.

When the compound reduces or inhibits the promoter activity, the compound may be used as an antipyretic
30 drug because the compound decreases or inhibits heat production.

A salt of the compound obtained using the screening method or the screening kit described above includes pharmaceutically acceptable salt. For example,
35 salts formed with inorganic bases, organic bases,

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Preferred salts formed with inorganic bases include alkaline metal salts such as sodium salts and potassium salts, alkaline earth metal salts such as calcium salts and magnesium salts, and aluminum salts and ammonium salts, etc.

Preferred salts formed with inorganic acids include salts formed with hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid, etc.

Preferred salts formed with basic amino acids include salts formed with arginine, lysine, and ornithine, etc., and preferred salts formed with acidic amino acids include salts formed with aspartic acid and glutamic acid, etc.

For example, the said compound or its salt can be orally administered as sugar coated tablet, capsule, elixir, and microcapsule, etc., or non-orally as

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may be combined with, for example, buffers (e.g. phosphate buffer, sodium acetate buffer), soothing agents (e.g. benzalkonium chloride, procaine hydrochloride), stabilizers (e.g. human serum albumin, polyethylene glycol), preservatives (e.g. benzylalcohol, phenol), and antioxidants. The preparation for injection is usually filled in appropriate ampoules.

The preparations obtained as described above are safe and low toxic, and can be administered to, for example, human and mammals (e.g. rats, mice, rabbits, sheep, pigs, cattle, cats, dogs, monkeys, etc.).

The dosage of the said compound or its salt differs depending on the target individual, target organ, symptoms, and administration method, etc. When it is administered orally, in general, for adults (60 kg body weight), about 0.1 - 100 mg per day, preferably about 1.0 - 50 mg per day, more preferably about 1.0 - 20 mg per day is administered. When it is administered non-orally, the dosage per dosing differs depending on the target individual, target organ, symptoms, and administration method, etc. For example, in case of injection in general, for adults (60 kg body weight), it is desirable to intravenously inject about 0.01 - 30 mg per day, preferably about 0.1 - 20 mg per day, more preferably about 0.1 - 10 mg per day. Converting the dosage for 60 kg, the said compound or its salt can be administered to other animals.

In this specification and drawings, the codes of bases and amino acids are according to IUPAC-IUB Commission on Biochemical Nomenclature or common codes in the art. The examples are shown below. For amino acids that may have the optical isomer, L form is presented unless it is specified.

DNA : deoxyribonucleic acid
 cDNA : complementary deoxyribonucleic acid

5 The SEQ ID NOs shown in the Sequence Listing of
this Specification present the sequences below.

[SEQ ID NO: 2] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[SEQ ID NO: 4] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

EXAMPLES

Escherichia coli transformant TOP10/pPCR-ucp2p5' #1-10 obtained in the Example 1 described below was deposited with the Ministry of International Trade and Industry, Agency of Industrial Science and Technology, National Institute of Bioscience and Human Technology (NIBH) as deposit number FERM BP-6587 on November 24, 1998 and with Institute for Fermentation, Osaka (IFO) as deposit number IFO 16219 on November 11, 1998.

Example 1 Cloning of human UCP-2 cDNA

Using 0.5 ng of human kidney cDNA (Clontech Laboratory, California, USA) as the template and the base sequence of base number 55 to 82: 5'-

ATGGTTGGGTCAAGGCCACAGATGTGCCC-3' of previously

ATACAGGCCGATGCGGACAGAGGCAAAGCT-3' as oligonucleotide

Using this plasmid DNA carrying the insert as the template, oligonucleotide primers were prepared, and probes were prepared using PCR DIG probe synthesis kit (Boehringer-Mannheim Co.) following the attached instruction. Using the prepared probes, human genomic DNA library (Clontech Laboratory, California, USA) in 3×10^6 phages was screened using nitrocellulose filters. Plaque hybridization was performed using DIG Easy hyb (Boehringer-Mannheim Co.), DIG Wash and Block Buffer Set (Boehringer-Mannheim Co.), and DIG nucleic acid detection kit (Boehringer-Mannheim Co.) following the attached instruction. As a result, eight positive clones were obtained from 3×10^6 phages. Of these clones, an inner primer of non-coding exon of previously reported human UCP-2 cDNA sequence [Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687] was synthesized (5'-CAAAGCTGCCAGTGGCTATCATGGCCCG-3'), and a clone containing the non-coding exon was detected by PCR using a primer containing EMBL3 sequence (5'-GACCGGTCGACCCAGATCTGGGTGCGACCTG-3'), and a genomic clone containing the 5' upstream region of UCP-2 was obtained. From the genomic clone, 3.5 kbp fragment containing UCP-2 promoter region was prepared, and inserted into pCR-blunt vector (Invitrogen Co.), and transformant *E. coli* TOP/10 pCR-UCP2P5' #1-10 was prepared. After that, the restriction enzyme map was prepared and the base

sequence was determined. The determined base sequence is shown in Figures 1 to 6. As shown in Figures 1 to 6, base number 2271 - 2326 and 3416 - 3505 were completely consistent with human UCP-2 cDNA (Gimeno, R.E. et al.

5 (1998), Diabetes, 47 (4), 685-687). Furthermore, the terminal base sequences of the consistent regions were consistent with Shahnborn rule, which is the characteristic of intron-exon boundary base sequence, suggesting that the consistent base sequences are
10 introns. A sequence likely to be CpG island (base number about 2070 - 2270), which is a characteristic of promoters without containing TATA-box sequence, was also confirmed upstream of the first exon. In the promoter sequence described above, PPRE (base number
15 284 - 296), which is the regulator sequence of promoters of fat cell-related genes, and three C/EBP binding sites (base number 1316 - 1320, 1364 - 1368, 1698 - 1692) were confirmed.

20 Example 2 Examination of human UCP-2 gene promoter activity

To confirm the promoter activity of the cloned genomic DNA fragment, luciferase assay was performed. pGL3-Basic plasmid (Promega Co.) carrying firefly
25 luciferase gene as the reporter gene was used for the vector. As the internal standard, pRL-SV40 plasmid (Promega Co.) expressing sea pansy luciferase under control of SV40 promoter was used.

~~EcoRI fragment (3.5 kb) was isolated from the
30 genomic human UCP-2 DNA and blunted using Blunting High Kit (TOYOBO Co.), and then ligated to SmaI-digested pGL3-Basic plasmid DNA. Following the above procedure, human UCP-2 promoter/luciferase vector (pGL-3UCP2) was constructed in which the base number 1 - 3505 shown in
35 Figures 1 to 6 was inserted into pGL3-Basic vector.~~

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The constructed human UCP-2 promoter/luciferase vector was transiently transfected in HepG2 cells, in which constant expression of UCP-2 was confirmed by RT-PCR, and the activity was examined.

5 HepG2 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 60,000 cells/well, and cultured at 37°C in 5% CO₂ overnight. Using SuperFect Transfection Reagent (QIAGEN Co.), cells were transiently transfected with 1 µg of human UCP-2
10 promoter/luciferase vector DNA or pGL3-Basic DNA and 0.1 µg of pRL-SV40 DNA. The procedure was performed according to the attached instruction. Then, the cells were cultured at 37°C in 5% CO₂ for 24 hours, and the luciferase activity was detected using PicaGene Dual
15 Sea Pansy (Nippon Gene Co.) according to the attached instruction. The measurement data were presented as relative activity to the internal standard value of pRL-SV40-derived sea pansy luciferase activity. The results are shown in Figure 7. The human UCP-2
20 promoter/luciferase vector-derived luciferase activity was markedly higher than that of pGL3-Basic lacking the promoter. Therefore, the genomic DNA of human UCP-2 gene of this invention has a promoter activity reflecting the in vivo UCP-2 gene expression system.

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Example 3 Examination of human UCP-2 gene promoter activity in human differentiated fat cell-like cells

Using the human UCP-2 promoter/luciferase vector DNA obtained in Example 2, the promoter activity in
30 human fat cell-like cells differentiated from MG-63 cells was confirmed.

MG-63 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 100,000 cells/well, and cultured at 37°C in 5% CO₂ overnight. Using SuperFect
35 Transfection Reagent (QIAGEN Co.), cells were

transiently transfected with 1 µg of human UCP-2 promoter/luciferase vector DNA or pGL3-Basic DNA and 0.1 µg of pRL-SV40 DNA. The procedure was performed according to the attached instruction. The culture medium was exchanged to Dulbecco's modified Eagle's MEM (Gibco Co.) containing 5% rabbit serum (Gibco Co.), and differentiation to fat cell-like cells was induced. Then, the cells were cultured at 37°C in 5% CO₂ for 24, 36, and 72 hours. After culture, the luciferase activity was detected in each culture as described in Example 2. The measurement data were presented as relative activity to the internal standard value of pRL-SV40-derived sea pansy luciferase activity. The results are shown in Figure 8. The human UCP-2 promoter/luciferase vector-derived luciferase activity was markedly higher than that of pGL3-Basic lacking the promoter in fat cell-like cells differentiated from human MG-63 cells. Therefore, the genomic DNA of human UCP-2 gene of this invention has the promoter activity reflecting the in vivo UCP-2 gene expression system in fat cell-like cells differentiated from human MG-63 cells.

Example 4 Preparation of human UCP-2 promoter-deficient vector

The human UCP-2 promoter/luciferase vector prepared in Example 2 was digested with KpnI and MluI, and the human UCP-2 promoter-deficient vector shown in Figure 9 was prepared using Deletion Kit for Kilo-Sequence (Takara Shuzo Co.) following the protocol. The plasmid digested with KpnI and MluI was purified by phenol extraction and ethanol precipitation. Then, the precipitated DNA was treated with exonuclease III and sampled every one minute, and the reaction was terminated. The samples were treated with Mung bean

nuclease and the ends were blunted. The ends were restored by Klenow fragment, and the DNA was circularized by DNA ligase. The circularized DNAs were re-treated with MluI to linearize plasmid in which deletion did not occur. *E. coli* JM 109 competent cells (Takara Shuzo Co.) were transformed with this reaction solution. The obtained deficient clone plasmids were purified by publicly known method. The molecular weights of the deficient plasmids were confirmed by agarose gel electrophoresis, and clones were selected. The base sequences of these clones were confirmed by publicly known method.

Using these plasmids, the promoter activity was measured by the procedure described in Example 2 (Figure 10).

When the base sequence containing PPRE (base number 284 - 296) shown in Example 1 was deleted, about 70% increase in the UCP-2 promoter activity was observed, suggesting that this sequence region has a UCP-2 promoter suppressor activity. When the base sequence containing the two C/EBP binding sites (base number 1316 - 1320 and 1364 - 1368) was deleted, about 30% of the UCP-2 promoter activity decreased, suggesting that the base sequence containing these two C/EBP binding sites has a UCP-2 promoter enhancer activity. When 290 bases were deleted from the transcriptional initiation site toward downstream, no UCP-2 promoter activity was detected. Therefore, the genomic DNA of human UCP-2 gene of this invention has a promoter activity reflecting the in vivo UCP-2 gene expression control system.

INDUSTRIAL APPLICABILITY

Since UCP-2 promoter of this invention contains the regulator sequence, it has higher activity

reflecting the in vivo UCP-2 DNA expression system in human than the promoter lacking the regulator sequence. Therefore, the UCP-2 promoter of this invention can be used as a promoter inserted in vectors for treatment of human diseases and setting drug-screening systems under conditions closer to in vivo environment in human.

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